(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 February 2003 (20.02.2003)

PCT

(10) International Publication Number
WO 03/013493 A1

(51) International Patent Classification[7]: A61K 31/166, A61P 19/02, 25/02, 29/00, 1/04, 9/10, 11/00, 11/06, A61K 31/165

(21) International Application Number: PCT/EP02/08379

(22) International Filing Date: 26 July 2002 (26.07.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
MI01A001733 7 August 2001 (07.08.2001) IT

(71) Applicant *(for all designated States except US)*: **ITALFARMACO S.P.A.** [IT/IT]; Viale Fulvio Testi, 330, I-20126 Milano (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MASCAGNI, Paolo** [IT/IT]; Via Dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **LEONI, Flavio** [IT/IT]; Via Dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **PORRO, Giulia** [IT/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **PAGANI, Paolo** [IT/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **DONA', Giancarlo** [IT/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **POZZI, Pietro** [IT/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **DINARELLO, Charles** [US/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **FANTUZZI, Giamila** [IT/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **SIEGMUND, Britta** [DE/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **REZNIKOV, Leonid** [US/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **BUFLER, Philip** [DE/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **KIM, Soo-Hyun** [KR/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT).

(74) Agents: **BANFI, Paolo** et al.; Bianchetti Bracco Minoja S.r.l., Via Rossini, 8, I-20122 Milano (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/013493 A1

(54) Title: HISTONE DEACETYLASE ENZYME-INHIBITING DERIVATIVES OF HYDROXAMIC ACID AS NEW CYTOKINE SYNTHESIS-INHIBITING ANTI-INFLAMMATORY DRUGS

(57) Abstract: The use of derivatives of hydroxamic acid having histone deacetylase enzyme-inhibiting activity for the preparation of anti-inflammatory medicaments is disclosed.

HISTONE DEACETYLASE ENZYME-INHIBITING DERIVATIVES OF HYDROXAMIC ACID AS NEW CYTOKINE SYNTHESIS-INHIBITING ANTI-INFLAMMATORY DRUGS

This invention relates to the use of hydroxamic acid derivatives having histone deacetylase enzyme-inhibiting activity for the preparation of anti-inflammatory medicaments.

Some derivatives of hydroxamic acid which inhibit histone deacetylases are known. Those which have been most extensively studied are suberoylanilide hydroxamic acid (SAHA), N-hydroxy-3-[3-(hydroxyamino)-3-oxo-1-propenyl]-benzamide (CBHA) and trichostatin (TSA). Other derivatives are described in Proc. Natl. Acad. Sci. USA 95, 3003-3007, 1998; Tumori, 2001 Nov-Dec, 87 (6): S12-4; Anticancer Drugs, 2002 Jan, 13 (1): 1-13; Nature Rev Cancer, 2001 Dec, 1 (3): 194-202; Curr Opin Oncol, 2001 Nov. 13 (6): 477-83; Cancer Chemother Pharmacol, 2001, Aug, 48 Suppl 1:S20-6; Cancer Chemother Pharmacol, 2001 Aug, 48 Suppl 1:S17-9; Haematologica, 2001 Sep; 86 (9): 908-17.

These compounds have mainly been studied as potential anti-tumoral drugs: trichostatin, an antifungal antibiotic isolated from *Streptomyces hygroscopicus*, is a potent inducer of murine erythroleukaemic cell differentiation (Cancer Res. 47, 3288-3691, 1987), while SAHA and CBHA have been studied by the Sloan Kettering Institute (WO 95/31977) as tumour cell differentiation inducing agents.

The therapeutic use of histone deacetylase inhibitors to treat tumours is described in Anticancer Res. 20, 1471-1486, 2000 and Exp.Opin.Invest. Drugs 8(10),1611-1621,1999.

It has now been found that the known derivatives of hydroxamic acid having histone deacetylase inhibiting activity, especially trichostatin and

SAHA, inhibit the synthesis of pro-inflammatory cytokines, and can therefore be used to treat disorders which can be alleviated by inhibiting those cytokines. Examples of such disorders, with an inflammatory and/or autoimmune basis, include multiple sclerosis, Crohn's disease and ulcerative colitis, atherosclerosis, rheumatoid arthritis, psoriasis, spondyloarthropathies (anchilosating spondilitis, psoriatic arthritis, arthritis connected to ulcerative colitis), AIDS-related neuropathies, asthma, chronic obstructive lung diseases, bronchitis, pleuritis, acute and chronic hepatitis (either viral, bacterial or toxic), acute glomerulonephritis and, broadly speaking, all disorders with an inflammatory component

For the therapeutic uses considered, the hydroxamic acid derivatives will be administered at doses ranging between 1 and 500 mg one or more times a day, depending on the disorder concerned and the pharmacotoxicological characteristics of the compound in question, which can be administered in the form of suitable oral, parenteral or topical formulations.

The following examples illustrate the invention in greater detail.

EXAMPLE 1- Inhibition of cytokine production *in vitro*

The treatment of lymphocytes with lipopolysaccharide (LPS) induces the production of various pro-inflammatory cytokines, such as TNFα, IL-1β and IFNγ (J. Biol. Chem. 1990; 265(18): 10232-10237; Science, 1998; 281:1001-1005).

The effect of SAHA and TSA has been studied by evaluating the inhibitory effect of the compound on cytokine production by peripheral blood mononuclear cells (PBMC) from healthy volunteers (2 to 6 donors), stimulated with LPS.

Samples of peripheral blood or buffy coats from healthy volunteers were used. The samples were separated by centrifugation on density gradient

using Ficoll-Hypaque, and the PBMCs thus obtained were seeded in 96-well dishes (500,000 cells/well), incubated for 60 minutes with SAHA or TSA at various doses, and then stimulated with LPS from *E. coli* O55:B5 (10 ng/ml) for 24 hours in the presence of the compound. At the end of the treatment the pro-inflammatory cytokines TNFα, IL-1β were measured by means of an electrochemiluminescence assay (ECL) using specific commercial antibodies.

Interferon γ (IFNγ) was measured with a commercially available ELISA assay.

Cytokine IFNγ is produced by the T lymphocytes following their stimulation by pro-inflammatory cytokines, especially IL-12 and IL-18 (Dinarello C. A. and Moldawer L. L. Proinflammatory and anti-inflammatory cytokines in rheumatoid arthritis. A primer for clinicians. 2nd Edition, Amgen Inc., 2000).

The effect of SAHA and TSA on IFNγ synthesis induced by stimulating PBMCs with IL-12 and IL-18 *in vitro* was evaluated on this basis. PBMCs were seeded in round-bottomed 96-well dishes (500,000 cells/dish), and incubated with various doses of SAHA or TSA for 60 minutes. At the end, the cells were stimulated for 48 hours in the presence of the compound by simultaneous addition of recombinant IL-12 (10 ng/ml) and recombinant IL-18 (20 ng/ml). The quantity of IFNγ produced was determined with a commercial ELISA assay.

The effect of the various doses of SAHA and TSA was measured as the percentage inhibition of production of the cytokine in question compared with untreated control cells. The concentration able to induce 50% inhibition of cell growth (IC_{50}) was determined by linear regression.

The results are summarised in the table below:

Cytokine	SAHA IC_{50} (nM)	TSA IC_{50} (nM)
TNFα	200	50
IL-1β	100	100
IFNγ	50	10
IFNγ (from IL-12 + IL-18)	740	490

These results clearly indicate that SAHA and TSA inhibit synthesis of all the inflammatory cytokines induced by LPS with an IC_{50} in the nanomolar range (50-200nM).

SAHA and TSA also inhibit the synthesis of IFNγ by the T lymphocyte cells, as demonstrated by their efficacy (IC_{50} 740 nM and 490 nM respectively) when the stimulus used was the combination of IL-12 and IL-18 specific for that cell line.

EXAMPLE 2 - Inhibition of cytokine production *in vivo*

Systemic administration of LPS to laboratory animals is known to induce rapid, massive production of pro-inflammatory cytokines (Immunopharmacol. 1992; 14(6): 1045-1050).

Female BALB/c mice (20-22 grams) were treated orally with SAHA at the various doses indicated, then treated after 60 minutes with LPS from *E. Coli* O55:B5 (30 mg/Kg intraperitoneally). 90 minutes after the endotoxin administration, blood samples were taken from all the treated animals (10 animals/group), and the cytokines were measured with commercial ELISA assays.

The results are set out in the table below, and expressed as the percentage inhibition of production of the cytokine in question:

TREATMENT	% inhibition of TNFα	Inhibition of IL-1β	Inhibition of IL-6
SAHA			
0.1 mg/Kg	40	13	10
1 mg/Kg	53	15	3
10 mg/Kg	67	35	7
25 mg/Kg	68	37	25
50 mg/Kg	not done	51	29

The above results indicate that SAHA is active orally and able to inhibit, to a dose-dependent extent, pro-inflammatory cytokine synthesis induced *in vivo* in the mouse by administering endotoxin, thus confirming the results obtained *in vitro*.

EXAMPLE 3. Con A-Induced Liver Injury.

C57B16 mice were injected i.p. with either water vehicle or SAHA and after 1 h were injected i.v. with Con A as described in Proc. Natl. Acad. Sci. USA, (2000), 97, 2367-2372. After 24 h, serum amino-alanine transferase was measured.

Intravenous injection of Con A results in hepatic cell death within 12-24 h with markedly elevated serum levels of hepatic enzymes such as alanine amino transaminase (ALT). In mice pretreated with a single dose of SAHA (50 mg/kg) given i.p. 1 h before Con A, the 24-h level of serum ALT (mean ± SE) was 8.144 ± 2.091 units/liter compared with 15.190 ± 2.580 in vehicle-treated mice (*n* =6 per group).

EXAMPLE 4. Nitric Oxide Production from Mouse Peritoneal Macrophages.

C57BLy6 mice were injected i.p. with 1 ml of sterile thioglycolate broth and killed after 5 days, and macrophages were isolated using instillation of 10 ml of ice-cold PBS into the peritoneal cavity. The cells were centrifuged (350 x g) and 3 ml of erythrocyte lysing reagent (PharMingen) was added for 10 min.

Seven milliliters of DMEM containing 5% FCS was added and the cells were centrifuged at 4°C. The cells were resuspended in DMEM at 1 million per ml and 0.5 ml were added to wells of a 48-well plate. SAHA was added for 60 min at 37°C and then stimulated with the combination of TNF-α plus IFN-γ. After 24 h, NO levels in the supernatant were determined using the Griess reagent as described in Am.J.Physiol.Cell Physiol. (2001), 280, C441-C450.

As shown in Fig. 1, SAHA inhibited NO production; at 200 nM, there was a 50% reduction *(P,* 0.05). Further reductions of 80 and 85% were observed at 400 and 800 nM, respectively.

EXAMPLE 5. Inhibition of IL-12 production by cultured monocytes.

Venous blood was obtained from consenting adults and separated over Ficoll-Hypaque. The PBMC fraction was washed and adjusted to five million cells per ml. Five hundred microliters was aliquoted into each well of 24-well flat-bottom plates, 100 ml of SAHA was added, and the plates were incubated for 1 h at 37°C. The cells were stimulated with LPS, soluble OKT3, or cytokines, and after 24 or 48 h At 37°C the supernatant was removed and frozen for cytokine assays. Monocytes were isolated by centrifugation over Percoll, washed, suspended in RPMI with 10% FCS, and aliquoted at 2 million cells per ml in Petriperm Teflon-coated culture dishes (Sigma). The ELISA for human IL-12 (p70) was purchased from Endogen (Woburn, MA).

As shown in Fig. 2, there was a dose-dependent reduction in LPS/IFN-γ-induced IL-12 production in nonadherent human monocytes. At 200 nM, the reduction was 55% *(P,* 0.01) and at 86% at 400 nM *(P,* 0.001).

EXAMPLE 6 Dextran-induced colitis

Female, 8 week-old C57BL/6 mice (The Jackson Laboratories, Bar Habor, ME) weighing 20-22 g were used in this study. The animals were housed in rooms at a controlled temperature and a 12 h day/night rhythm.

They were fed standard mice chow pellets ad libitum, had free access to tap water supplied in bottles, and were acclimatized to the conditions at least seven days before they were used in experiments. Mice were killed by cervical dislocation under isoflurane anesthesia (Fort Dodge, Iowa City, IA).

Mice were fed 3.5% dextran sulfate sodium (DSS; molecular weight 40 kDa; ICN, Aurora, OH) dissolved in sterile, distilled water ad libitum from day one to five followed by a five day observation period. SAHA was administered once daily orally (p. o.) in a total volume of 200 µl and a concentration of 10 mg/kg body weight (BW) throughout the experiment (day 1 to 10). Control mice had free access to water and received either SAHA (10 mg/kg BW) or water p. o. once daily for 10 days.

Body weights, occult blood or the presence of gross blood per rectum, and stool consistency were determined daily. Two investigators blinded to the protocol assessed the clinical score (table 1). Weight loss < 1% compared to day 1 was counted as 0 points, weight loss of 1 to < 5% as 1 point, 5 to < 9.9% as 2 points, 10 to 20% as 3 points and more than 20% as 4 points. For stool consistency, 0 points were given for well-formed pellets (formed), 2 points for pasty and semi-formed stools which did not stick to the anus (soft), and 4 points for liquid stools that did stick to the anus (diarrhea). Bleeding was scored 0 points for no blood in hemoccult, 2 points for positive hemoccult, and 4 points for gross bleeding. These scores (body weight, stool consistency, rectal bleeding) were added and divided by 3 resulting in a total clinical score ranging from 0 (healthy) to 4 (maximal activity of colitis).

Post mortem (on day 10), the entire colon was removed from the caecum to the anus and the colon length was measured as an indirect marker of inflammation. Colon length has been shown to be a reliable parameter in this model as DSS-induced colitis is associated with colon shortening as described previously [Gastroenterology,1990, 98,:694:J. Pharmacol.Exp.Ther.,

2001, 296:99-105].

From the results, reported in the following Tables 1-6, it is evident that SAHA effectively counteracts dextran-induced colitis, a valid and established model of inflammatory bowel diseases in humans.

Table 1. **Clinical activity score [Lab. Invest., 1993, 69:238-249].**

Score points	Weight loss	Stool consistency	Rectal bleeding
0	0%	Formed	Negative hemoccult
1	(>0%) <5%		
2	5-9.9%	Soft	Positive hemoccult
3	10-20%		
4	>20%	Diarrhea	Macroscopic bleeding

Table 2. Weight

	Days									
Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
DSS + SAHA	19.1±0.2	18.8±0.2	19.1±0.3	18.4±0.4	18.4±0.4	18.7±0.3	18.0±0.4	16.9±0.3	16.6±0.4	16.6±0.4
DSS + water	18.7±0.5	19.0±0.5	19.4±0.5	18.9±0.5	18.9±0.5	18.8±0.6	18.8±0.6	16.6±0.5	16.8±0.5	16.8±0.5
SAHA	18.9±0.3	18.8±0.4	18.9±0.2	18.9±0.3	19.0±0.1	19.0±0.3	18.9±0.1	19.0±0.3	19.1±0.1	19.1±0.2
Water	19.0±0.2	19.0±0.3	18.9±0.1	19.0±0.2	19.0±0.4	19.0±0.2	19.1±0.2	19.1±0.1	19.1±0.3	19.1±0.1

Table 3. Stool consistency as score

	Days									
Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
DSS + water	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.4	1.2 ± 0.4	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	1.6 ± 0.4	1.6 ± 0.4
DSS + SAHA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.4	0.8 ± 0.4	2.0 ± 0.0	2.0 ± 0.0	1.2 ± 0.4	1.2 ± 0.4
SAHA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Water	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 4. Bleeding

	Days									
Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
DSS + water	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.4	1.6 ± 0.4	2.8 ± 0.4	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	1.6 ± 0.4
DSS + SAHA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.2 ± 0.4	2.4 ± 0.4	2.0 ± 0.0	1.6 ± 0.4	1.2 ± 0.4	0.8 ± 0.4
SAHA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Water	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 5. Complete clinical score

	Days									
Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
DSS + water	0.0 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.8 ± 0.1	1.1 ± 0.2	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	1.9 ± 0.2
DSS + SAHA	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.7 ± 0.3	1.3 ± 0.3	1.5 ± 0.1	1.9 ± 0.1	1.5 ± 0.4	1.3 ± 0.4
SAHA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
Water	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

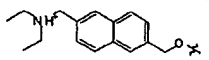
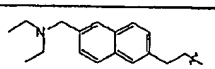
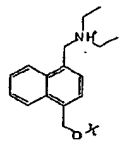
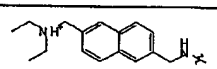
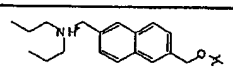
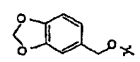
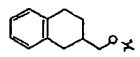
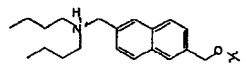
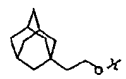
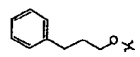
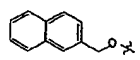
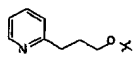
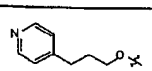
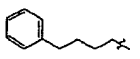
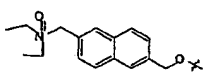
Table 6. Colon length

Group	Colon length (cm)
DSS + water	7.6 ± 0.2
DSS + SAHA	9.2 ± 0.2
SAHA	10.1 ± 0.2
Water	10.5 ± 0.3

EXAMPLE 7

A series of hydroxamic acid derivatives described in EP 901465 were subjected to the histone deacetylase (HDAC) and TNFα inhibition tests in accordance with the methods descried by Lechner et al., Biochim Biophys. Acta 1996, 1296, 181-188 and Moreira A.L. et al., J. Exp. Med. 1993, 177, 1657-1680 respectively.

The results, set out in the following table, show that a linear correlation exists between the ability of these compounds to inhibit the synthesis of TNFa and their inhibition of HDAC activity.

	General structure	HDAC		TNF	
	R	IC50 nM	Potency	IC50 nM	Potency
1		20,0	100,00	7,0	100,00
2		62,0	32,26	10,2	68,75
3		65,0	30,77	10,3	68,02
4		78,0	25,64	11,2	62,66
5		46,7	42,86	12,7	55,28
6		80,0	25,00	50,0	14,00
7		91,0	21,98	65,5	10,68
8		133,3	15,00	67,8	10,32
9		600,0	3,33	159,1	4,40
10		105,0	19,05	159,1	4,40
11		8,1	246,91	159,1	4,40
12		260,0	7,69	230,0	3,04
13		260,0	7,69	270,0	2,59
14		66,7	23,08	300,0	2,33
15		206,7	9,68	1000,0	0,70

CLAIMS

1. The use of hydroxamic acid derivatives having histone deacetylase inhibiting activity for the preparation of anti-inflammatory medicaments.

2. Use as claimed in claim 1, wherein the derivatives are selected from suberoylanilide hydroxamic acid (SAHA), N-hydroxy-3-[3-(hydroxyamino)-3-oxo-1-propenyl]-benzamide (CBHA) and trichostatin (TSA).

3. Use as claimed in claim 2, wherein the derivatives are selected from suberoylanilide hydroxamic acid (SAHA) and trichostatin (TSA).

4. Use as claimed in claim 1 or 2 for the preparation of medicaments for the treatment of multiple sclerosis, Crohn's disease and ulcerative colitis, atherosclerosis, rheumatoid arthritis, psoriasis, spondyloarthropathies (anchilosating spondilitis, psoriatic arthritis, arthritis connected to ulcerative colitis), AIDS-related neuropathies, asthma, chronic obstructive lung diseases, bronchitis, pleuritis, acute and chronic hepatitis (either viral, bacterial or toxic), acute glomerulonephritis.

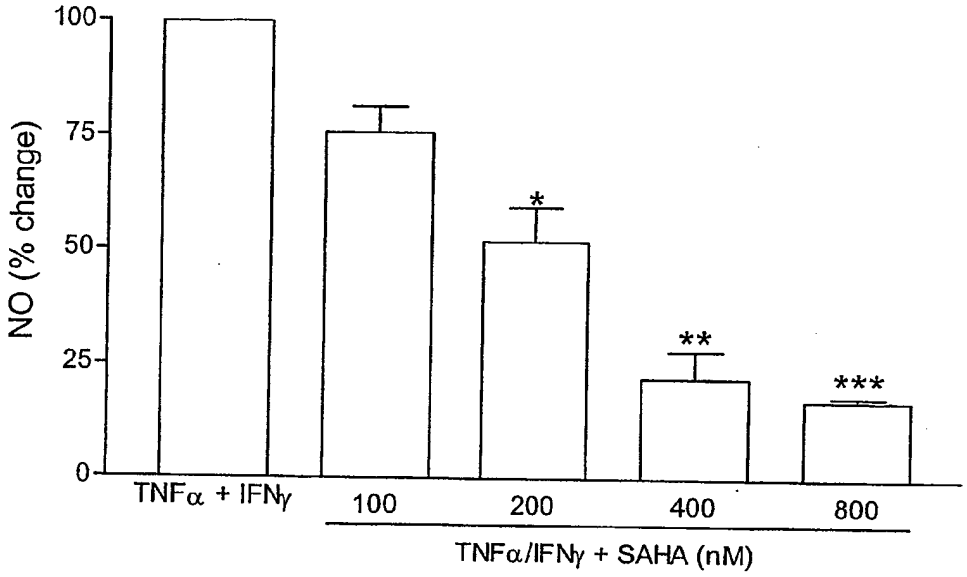

100
75
50
25
0
NO (% change)
*
**
***
TNFα + IFNγ
100
200
400
800
TNFα/IFNγ + SAHA (nM)

Fig. 1

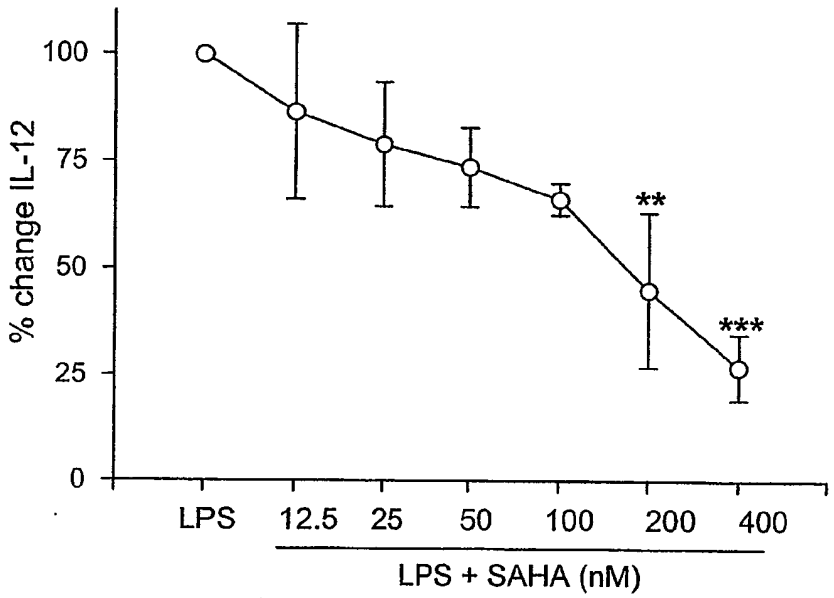


Fig. 2

INTERNATIONAL SEARCH REPORT

Internatic _ pplication No
PCT/EP 02/08379

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/166 A61P19/02 A61P25/02 A61P29/00 A61P1/04
A61P9/10 A61P11/00 A61P11/06 A61K31/165

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, WPI Data, PAJ, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 35990 A (JAMISON TIMOTHY F ;HARVARD COLLEGE (US); TAUNTON JACK (US); HASSIG) 2 October 1997 (1997-10-02) page 11, line 5-8; figure 1A; example 1 page 82, line 14 -page 83, line 9 ---	1-4
X	HUANG N ET AL: "INHIBITION OF IL-8 GENE EXPRESSION IN CACO-2 CELLS BY COMPOUNDS WHICH INDUCE HISTONE HYPERACETYLATION" CYTOKINE, ACADEMIC PRESS LTD, PHILADELPHIA, PA, US, vol. 9, no. 1, January 1997 (1997-01), pages 27-36, XP001013211 ISSN: 1043-4666 abstract page 28, column 1, line 14-17 --- -/--	1-4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 January 2003	15/01/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Ansaldo, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP 02/08379

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	MISHRA NILAMADHAB ET AL: "Histone deacetylase inhibitor Trichostatin A as a strong candidate for treatment of systemic lupus erythematosus." FASEB JOURNAL, vol. 15, no. 5, 8 March 2001 (2001-03-08), page A1214 XP009002039 Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001;Orlando, Florida, USA; March 31-April 04, 2001 ISSN: 0892-6638	1-4
Y	abstract	1-4
Y	RICHON VICTORIA M ET AL: "A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 6, 17 March 1998 (1998-03-17), pages 3003-3007, XP001120542 March 17, 1998 ISSN: 0027-8424 abstract; table 1	1-4
P,X	WO 02 055017 A (MISHRA NILAMADHAB ;KAMMER GARY M (US); UNIV WAKE FOREST (US)) 18 July 2002 (2002-07-18) page 6, line 7-11; claims 1,3,5,6,10-16 page 6, column EMB-2002, line 20 -page 7, line 17	1-4
X	EP 0 574 758 B (HOFFMANN LA ROCHE) 22 December 1993 (1993-12-22) page 2, line 41-43; claims 1,23	1,4
X	EP 0 757 984 B (ONO PHARMACEUTICAL CO) 12 February 1997 (1997-02-12) claim 7	1,4
X	WO 97 43251 A (BERTOLINI GIORGIO ;PAVICH GIANFRANCO (IT); BIFFI MAURO (IT); ITALF) 20 November 1997 (1997-11-20) page 2, line 3 - line 7; claims 1,4,6	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internatio pplication No
PCT/EP 02/08379

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9735990 A	02-10-1997	AU 2990597 A	17-10-1997
		WO 9735990 A2	02-10-1997
WO 02055017 A	18-07-2002	WO 02055017 A2	18-07-2002
EP 0574758 B	22-12-1993	AT 170840 T	15-09-1998
		AU 659555 B2	18-05-1995
		AU 3981693 A	16-12-1993
		BG 61724 B1	30-04-1998
		BG 97857 A	15-11-1994
		CA 2098168 A1	12-12-1993
		CN 1083062 A ,B	02-03-1994
		CZ 9301081 A3	16-02-1994
		DE 69320869 D1	15-10-1998
		DE 69320869 T2	29-04-1999
		DK 574758 T3	07-06-1999
		EP 0574758 A1	22-12-1993
		ES 2121896 T3	16-12-1998
		FI 932692 A	12-12-1993
		HR 930978 A1	30-04-1997
		IL 105921 A	04-01-1998
		JP 2039885 C	28-03-1996
		JP 6065196 A	08-03-1994
		JP 7076210 B	16-08-1995
		MX 9303391 A1	30-06-1994
		NO 932117 A	13-12-1993
		NZ 247765 A	27-11-1995
		PH 30245 A	05-02-1997
		PL 299261 A1	10-01-1994
		RO 112613 B	28-11-1997
		SI 9300289 A	31-12-1993
		SK 57393 A3	11-05-1994
		US 5318964 A	07-06-1994
		US 5447929 A	05-09-1995
		ZA 9303957 A	13-12-1993
EP 0757984 B	12-02-1997	AT 226936 T	15-11-2002
		DE 69624536 D1	05-12-2002
		EP 0757984 A1	12-02-1997
		JP 9104672 A	22-04-1997
		KR 231230 B1	15-11-1999
		US 6022893 A	08-02-2000
WO 9743251 A	20-11-1997	IT MI960968 A1	14-11-1997
		AU 713300 B2	25-11-1999
		AU 2896497 A	05-12-1997
		BR 9709234 A	10-08-1999
		CA 2254066 A1	20-11-1997
		CN 1221403 A	30-06-1999
		CZ 9803667 A3	16-06-1999
		DE 69703207 D1	02-11-2000
		DE 69703207 T2	01-02-2001
		DK 901465 T3	18-12-2000
		WO 9743251 A1	20-11-1997
		EP 0901465 A1	17-03-1999
		ES 2151267 T3	16-12-2000
		GR 3035128 T3	30-04-2001
		JP 2000510472 T	15-08-2000

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP 02/08379

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9743251 A		KR 2000010982 A	25-02-2000
		PL 329873 A1	12-04-1999
		PT 901465 T	31-01-2001
		RU 2177473 C2	27-12-2001
		SK 157998 A3	13-04-1999
		US 6034096 A	07-03-2000